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Development and Anatomy of *Nitidiscala tincta* (Carpenter, 1865) (Gastropoda: Epitoniidae)

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Abstract. The epitoniids (Gastropoda: Ptenoglossa) are one group for which there are no current well-supported hypotheses of relationships. Herein I describe the development and adult anatomy of an epitoniid, *Nitidiscala tincta,* and discuss intra-familial variation in characters used for higher level systematics. Female *N. tincta* lay 75 μm eggs in clusters in sand-covered capsules connected together along a mucus strand. The first two cleavages are synchronous, appear equal, and produce a polar lobe. Gastrulation is by epiboly and produces a trochophorelike stage with embryonic kidneys. At hatching the larvae are 125 μm long and have a small velum, heart, eyes, and a well-developed, black pigmented mantle organ. The shell is right-handed and slightly hydrophobic. The mantle cavity contains a gill with triangular lamellae, purple hypobranchial gland, and a triple-ridged osphradium. The acrembolic probosis ends in a robust pair or jaws slightly anterior to the split odontophore, a ptenoglossan radula, and two pairs of salivary glands. The presence of both caenogastropod and heterobranch developmental synapomorphies suggests that ideas about the evolution of development among gastropods may need to be reevaluated.

INTRODUCTION

Recent interest in high level gastropod systematics has focused on the relationships among well-known, purportedly monophyletic groups, using either exemplar species or composites of several disparate species (Haszprunar, 1988; Mikkelsen, 1996; Ponder & Lindberg, 1997). Some poorly known groups like deep-sea limpets and lower heterobranchs have also received considerable attention due to their perceived phylogenetic importance and the availability of recent detailed anatomical studies (Haszprunar, 1988; Healy, 1993; Ponder & Lindberg, 1997). However, there are still many groups whose relationships are not well understood. The Epitoniidae is one such group. What little is known about the biology of epitoniids has been used to group them with either heterobranchs (Gosliner, 1981; Habe & Kosuge, 1966; Robertson, 1985) or caenogastropods (Fretter & Graham, 1994; Haszprunar, 1985).

Robertson (1983a b, 1985) used reproductive and developmental data to argue for affinities of epitoniids and groups now considered to be lower heterobranchs. He pointed out that *Epitonium* Röding, 1798, species possess two putative heterobranch synapomorphies (Robertson, 1985): they lay eggs that are connected by chalazae (Robertson, 1973; but see Robertson, 1985 for a different opinion), and their larvae have pigmented mantle organs (PMO). Robertson (1983b) also reported that the larvae of *E. albidum* (d'Orbigny, 1842) have hydrophobic larval shells, another characteristic of heterobranchs (Collin, 1997).

Current opinion favors a close relationship between epitoniids and caenogastropods based on anatomical and ultrastructural similarities (Haszprunar, 1985; Healy, 1994; Ponder & Lindberg, 1997). Published discussions of high-level gastropod systematics group epitoniids with janthinids in the Janthinoidea (synonym: Epitonioidea; see Ponder & Warén, 1988), because members of these families share a ptenoglossan radula, an accessory pair of salivary glands, feed on cnidarians, and produce a purple secretion when disturbed (Fretter & Graham, 1994). The protoconchs are also strikingly similar. The Janthinoidea are grouped with the Triphoridae, Triforidae, Cerithiopsidae, Aclidae, and Eulimidae, as the Ptenoglossa on the basis of characters listed in Table 1. In recent phylogenetic analyses of the Gastropoda, a composite triphorid was used to represent the Ptenoglossa (Ponder & Lindberg, 1996, 1997). However, Ponder & Lindberg (1997) stated that the Ptenoglossa is "an almost certainly paraphyletic or polyphyletic taxon." This hypothesis cannot yet be tested because complete anatomical studies of most ptenoglossan groups are not currently available.

The characters listed in Table 1 are not optimal for

Table 1

Taxonomic distribution of characters traditionally used to support the "ptenoglossa."

Characters*	Epitoniidae	Janthinidae	Eulimidae	Cerithiopsidae	Triphoridae
Aphalic males	yes/no	yes	no	yes	yes
Spermatozeugmata	yes	yes	no	yes	yes
Protandrous hermaphrodites	yes	yes	some	no	no
Ptenoglossiate radula	yes	yes	yes	taenoglossan	sometimes
Divided radula/odontophore	yes	yes	?	no	no
Stylets	yes	yes	no	no	no
Medial tooth	yes/no	no	no	yes	yes
Adult purple gland	yes	yes	no	no	no
Larval PMO	yes	yes	no	no	no
Acrembolic proboscis	yes	no	yes	yes	yes
Cuticularized esophagus	yes	yes	yes	yes	no
Zygoneury	yes/no	yes	yes	?	no
Osphradium ridges	1 or 3	1 or 2	3 or 1	?	1
Open pallial oviduct	yes	no	yes/no	yes	yes
Pairs of salivary glands	2	2	1	1	1
Esophageal glands	no	no	no?	maybe	maybe
"Beaked" larval shell	no	no	no	yes	yes
Statocysts	yes	no	yes	?	?

* Characters are coded based primarily on the following papers. Epitoniids: Bouvier 1886; Healy 1994; Taki 1956, 1957; Thiele 1928. Janthinids: Bouvier 1886; Graham 1965; Healy 1994; Laursen 1953; Thiele 1928; Wilson & Wilson 1956. Eulimids: Warén 1983a, b. Triphorids: Fretter 1951; Healy 1990; Kosuge 1966; Marshall 1983. Cerithiopsids: Fretter 1951; Healy 1990.

phylogenetic analysis for several reasons. First, the variation within each group has not been investigated and may not be represented adequately. For example, most accounts of epitoniid radulae state that the central tooth is absent. Examination of *Couthouyella* Bartsch, 1909, however, shows that this is not the case in all epitoniids (Warén, 1980). Second, because so little is known about any one species, each group is coded as a composite of characters from many species that may not be closely related. Finally, homology assessments may based on very little comparative information. For example, both epitoniids and janthinids clearly have two pairs of salivary glands, whereas most caenogastropods have only one. However, cerithiopsids have been described as possessing a single pair (Fretter, 1951) in which one gland connects to the anterior esophagus and the other connects to the posterior esophagus. The morphology and ultrastructure of the two glands are different. This condition is interpreted as being a single pair in which the two glands have differentiated from each other (Fretter, 1951), but it could also represent a condition in which there were two pairs of glands with subsequent loss of one gland in each pair. Interpretation of osphradial morphology illustrates a similar situation: epitoniids and janthinids have either a single simple ridge or multiple parallel ridges. *Janthina* Röding, 1798, species have either one or two ridges, and epitoniids are reported to have one or three ridges. Triphorids, however, have a single thick ridge with a medial groove (Kosuge, 1966). It is unclear if this condition should be interpreted as a different character state or an intermediate form. Examination of more species will significantly increase our ability to refine these characters and to make well-informed homology assessments.

Herein I examine the development and adult morphology of *Nitidiscala tincta* (Carpenter, 1865). This study was undertaken (1) to provide the first description of early development for a member of this family, and (2) to provide descriptive morphological data that can be used as a basis for comparative and phylogenetic studies of epitoniids or Ptenoglossa.

MATERIALS AND METHODS

Adult *Nitidiscala tincta* were collected from the midintertidal zone near Santa Barbara, California (from Alegria [34°28′N, 120°17′W] and Coal Oil Point [34°05′N, 120°10′W]) in July 1997. They were collected by hand during low tide from sand around the base of the anemone *Anthopleura elegantissima* (Brandt 1835). Species were identified using DuShane (1979), and vouchers are deposited at the Field Museum of Natural History in Chicago (FMNH 282448). Animals were kept at ambient sea temperature (16–18°C) in custard dishes, with a layer of sand and one or several host anemones. Feeding behavior was observed under a dissecting microscope. The water was changed and the eggs were collected daily. Egg strings were kept in glass custard dishes or large petri dishes until the embryos hatched. One 11 mm-long female was kept alone to determine if females can store sperm.

Embryos were collected by pulling apart the capsules with forceps. Developmental stages were observed and

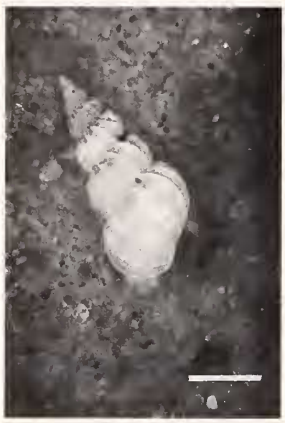

Figure 1

Adult *N. tincta.* Scale bar = 4 mm.

Figure 2

Egg capsules of *N. tincta.* Scale bar = 1 mm.

photographed every hour during cleavage and every day thereafter, at a total magnification of 100–200× with an Olympus compound microscope. Upon hatching, the larvae were measured and transferred to custard dishes with a density of about 1–2/10 ml. They were fed the unicellular brown alga *Isochrysis galbana,* and the water was changed every other day. Larvae were maintained this way for 2 weeks, but no further attempt was made to raise them to settlement.

Standard Prussian blue staining (Clarke, 1973) was used to detect embryonic albumin uptake. Embryos were carefully excapsulated and incubated in a solution of ferritin in seawater for 1–2 hours. They were then relaxed, fixed in 10% formalin, rinsed, and stained with HCl and potassium ferrocyanide solutions. In the presence of iron (from the ferritin) a blue product is formed. Staining was clearly visible with whole mount light microscopy. A negative control, for which the embryo was placed into a solution of seawater instead of ferritin was used in all staining experiments.

Adult animals were fixed in formalin and decalcified prior to staining with toluidine blue and dissection under a Wild M4 dissecting microscope. Tissues for SEM observations were dissected from fixed, decalcified animals, dehydrated in ethanol followed by Hexamethyldisalizine, and viewed with an Almray scanning electron microscope.

RESULTS

Adult *N. tincta* (Figure 1) are abundant on large patches of *Anthopleura elegantissima* in the mid-intertidal zone (Breyer, 1982). They occur amongst closely packed anemones buried in the sand, or occasionally stuck to an anemone's column wall by spirocysts. In the laboratory, snails usually remained buried completely in the sand unless they were feeding. Adults were attached to the substrate, their egg strings, and each other by a sticky mucus attachment thread secreted from the posterior sole of the foot. Attachment threads of several individuals were often entangled. Egg strings were commonly attached to anemones in the field, and egg strings from several females were usually deposited in a single tangle. Anemones do not respond when they contact the egg strings with their tentacles or oral disk.

Development

Female *N. tincta* lay egg strings which consist of multiple sand agglutinated capsules (Figure 2) arranged along a mucus thread, which is similar to and often connected to the attachment thread. Each capsule consists of a tight cluster of eggs surrounded by a capsule of tightly packed sand grains held together by a thin layer of mucus. The mucus thread which connects the capsules passes through the middle of each capsule, and the sand, eggs, and mucus can be removed without damaging the thread. In the laboratory, the isolated female continued to lay fertilized eggs for at least 16 days without reduction in mean eggs/capsule or egg viability. On average this female laid one egg string with 149 capsules every 2 days.

There were 10–60 eggs per capsule (mean = 32.9, SD = 13.1, n = 80 capsules, five each from 16 egg strings from different mothers). The number of eggs per capsule did not vary within an egg string, but did vary significantly among strings from different females (one-way ANOVA: $F = 50.3$, $df = 15$, $p < 0.001$). The mean outer diameter of 32 capsules from three females was 1.02 mm (SD = 0.157) and also varied among strings from different females (one-way ANOVA: $F = 52.2$, $df = 2$, $p < 0.001$). The small 75.3 μm (n = 62 from five females, SD = 1.7) white eggs were deposited while the germinal vesicle was still visible as a clear area in the otherwise opaque cytoplasm. Egg size did not vary among capsules, egg strings, or females. All eggs within a capsule devel-

Table 2

Developmental time table for *Nitidiscala tincta* at 16–18°C

Age	Stage
7 hours	First cleavage.
9 hours	2-cells.
10–11 hours	4-cells.
12 hours	3rd cleavage.
24 hours	Late cleavage.
2–3 days	Gastrulation.
3.5–4 days	Gastrulation complete, mouth visible, some slight ciliation.
4.5 days	"Trochophore" stage: Velum anlagen visible, larval kidneys.
5.5 days	Some shell growth, foot and operculum visible.
7 days	Early veliger morphology: Shell sculpture, long velar cilia, black PMO, and statocysts visible.
9 days	Dark color on shell sutures appears.
11 days	Heart beats slowly; eyes and right tentacle are visible.
12 days	Hatching at 125 μm.

oped synchronously, but the developmental stages were slightly staggered along an egg string.

After the germinal vesicle breaks down, the eggs have a uniformly granular appearance and two polar bodies are extruded. A developmental schedule of the following events is given in Table 2. The first two cleavages are both synchronous, appear to be equal, and produce a small polar lobe (Figure 3A). The third cleavage is difficult to see and produces four clear micromeres that are close to the size of the macromeres (Figure 3B). Subsequent cleavages are not quite synchronous. Although the membrane between the macromeres and micromeres is difficult to see, one of the macromeres rounds up and compacts slightly out of phase with the other macromeres. This asynchrony may begin as early as the third cleavage. Subsequent cleavage produces a round blastula that gastrulates by epiboly (Figure 3C): The clear micromeres slowly grow around the more opaque yolky macromeres. There is no invagination. After gastrulation, the embryo is slightly elongate, with a mouth about one-third of the way behind the apical tuft. A small ridge, the head-foot anlage, forms anterior to the mouth, and two small, clear embryonic kidneys appear lateral to the mouth (Figures 3D, E). The kidneys are equal in size and both take up ferritin. At this stage the embryo is slightly ciliated. The head-foot anlage gradually differentiates into a velar ridge and the foot, while the shell and operculum develop. After 8 days the statocysts are well developed, and the black PMO become visible on the dorsal right side, just posterior to the velum. Two areas of reddish pigment also develop on the shell, along the suture. At no stage is there a head vesicle. After 11 days the heart beats weakly, both eye spots are present, and the right tentacle is distinct. However the viscera is still an indistinct yolky mass. At this stage, Prussian blue staining shows that the left embryonic kidney has become much smaller and is difficult to detect in whole mounts, but still takes up ferritin. The only structure to stain on the right side is the PMO, which stains strongly in both the ferritin treatment and the negative control. The strong staining of the PMO might obscure the right embryonic kidney, if it is still functional.

Planktonic feeding larvae hatch after 13 days with a shell length of 124.7 μm (n = 89 from seven females, SD = 3.3; Breyer's [1982] statement that they hatch at 72 μm is in error) (Figure 3F). The shell is right handed. The velum and foot are small and pigmentless and the viscera are still not clearly differentiated, although there is a clear muscle attachment at the shell apex. The eyes and heart are well developed, but the tentacles are small. The velum does not project much from the body wall and is made up of few large cells, similar in appearance to those of vetigastropod veligers. The larval shells are hydrophobic and occasionally become trapped in the surface tension, although larvae generally remain near the bottom of the culture dish. *Nitidiscala tincta* veligers have been reported to live in culture for at least 2 months without settling (Smith & Breyer, 1983). Because animals were not raised through metamorphosis it was not possible to determine if the larval PMO is homologous to the adult hypobranchial gland as discussed by Robertson (1983b, 1985).

Feeding

Snails of all sizes (5–14 mm) were observed feeding on anemones in the laboratory. The proboscis is everted slowly until the expanded distal end is fully extended (Figure 4). The fully everted proboscis is approximately 1.5 times the length of the shell. The jaws, which are visible through the transparent wall of the proboscis, lie in the distal expansion of the extended proboscis. The extended proboscis waves around and probes the anemone. When the mouth contacts the end of a tentacle, it slips around the tentacle and works its way toward the base of the tentacle, engulfing it (Figure 4). There is a faint pumping motion on the ventral side of the proboscis just posterior to the distal expansion, and the engulfed tentacle appears to shrink a little. Then with a sudden smooth motion the distal end of the proboscis contracts laterally and severs the tentacle along the line of the tooth plates. The severed tentacle can be seen through the proboscis wall, moving toward the snail's head, and the proboscis quickly retracts. The anemone shows a clean diagonal incision on the tentacle stub where the tentacle was removed. Although snails usually engulf tentacles from the end, they sometimes surround them in the middle, folding them in half as the proboscis covers them,

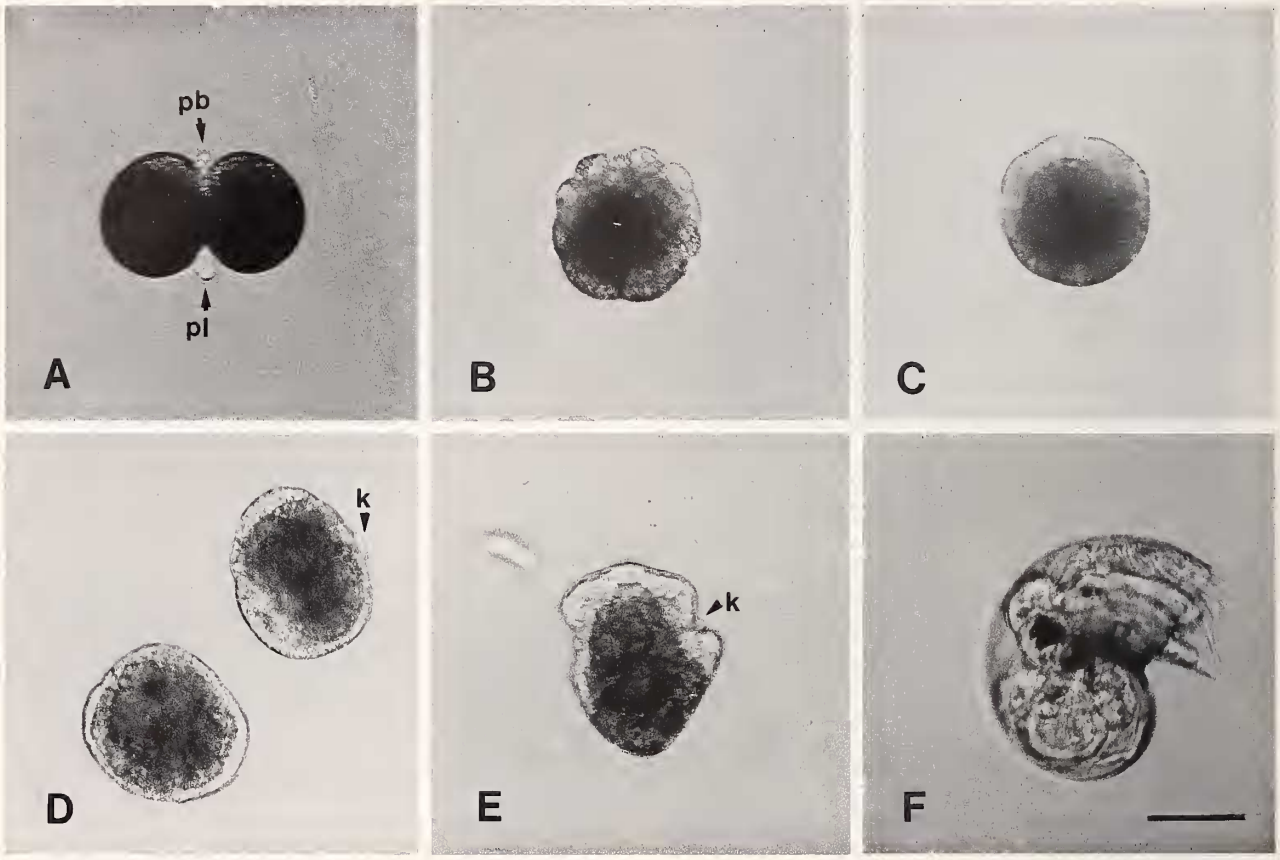

Figure 3

Developmental stages of *N. tincta*. A. Two-cell stage showing the polar bodies at the animal pole and the polar lobe at the vegetal pole. B. Later cleavage showing that the micromeres and macromeres are similar in size. C. Gastrula, after cell compaction. D. Early "trochophore" stage, showing the mouth and the embryonic kidneys. E. Late "trochophore" stage showing embryonic kidneys. F. Hatchling larva showing the distinct black PMO. Key: k = embryonic kidneys, pb = polar bodies, pl = polar lobe. Scale bar = 40 μm.

and sometimes they attack the tubercles on the anemone's column. In all cases that I observed, the snails attached the proboscis to some projection of the anemone's body wall. At no time did I observe the snails tearing bits from the anemone, engulfing them whole, or inserting the proboscis into the a hole in the anemone's body wall as has been reported in other descriptions of epitoniid feeding (den Hartog, 1987; Perron, 1978; Robertson, 1963, 1983b; Salo, 1977; Thorson, 1957). Such differences in feeding biology may be associated with the anatomical variation discussed below.

Morphology

External Morphology. The *N. tincta* collected for this study ranged in shell length from 5–14 mm. The shells are white with a distinctive purple marking overlying the hypobranchial gland along the top of the body whorl. The operculum is brown, uncalcified, ovate, and coiled. The visible body is white, with a rectangular foot, short tapered tentacles with an eye positioned dorso-laterally at the base of each. The snails are often attached to egg strings, anemones, or the substrate by a sticky string that originates in a medial groove that runs along the ventral side of the foot. The proboscis opening lies between and slightly below the tentacles. The viscera extend about four or five whorls into the shell. When removed from the shell, the dark coloration of the hypobranchial gland, the gills, and the osphradium can be seen clearly through the mantle.

Mantle Cavity. The mantle cavity is similar to those of other coiled caenogastropods and extends one whorl back from the aperture. The gill lamellae are triangular and the gill extends posteriorly to the end of the mantle cavity (Figure 5). Dorsal and parallel to the gill is the hypobranchial gland, a long ridge of large cells full of a purple exudate, which extends back about 75–85% of the first

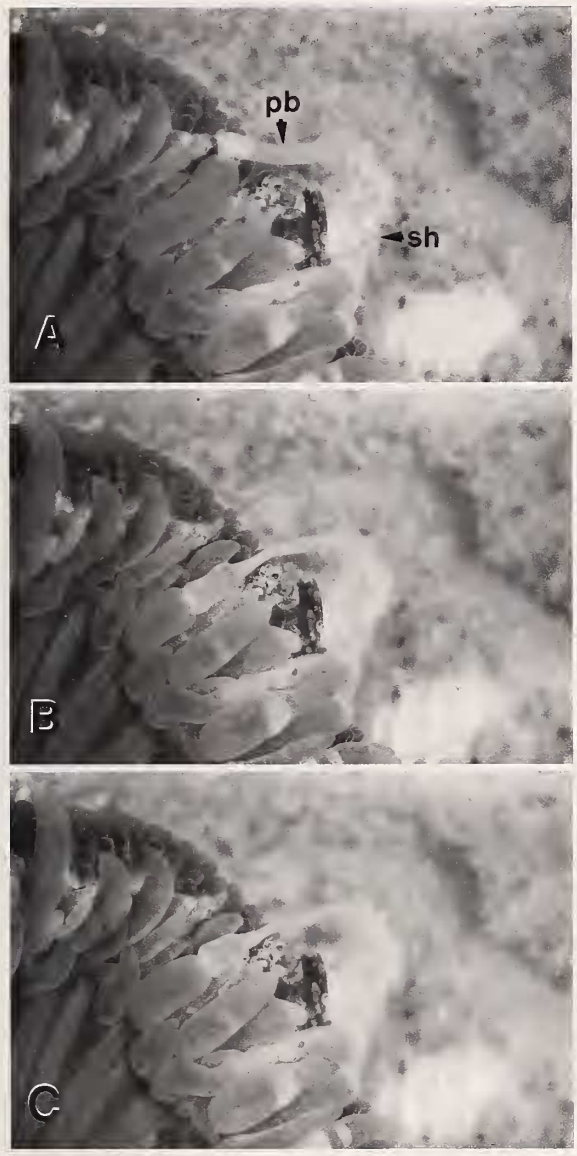

Figure 4

Adult *N. tincta* feeding on anemone. A. The proboscis probes the anemone, and B. slips around a tentacle. C. The proboscis contracts to cut the tentacle. Key: sh = shell, pb = proboscis.

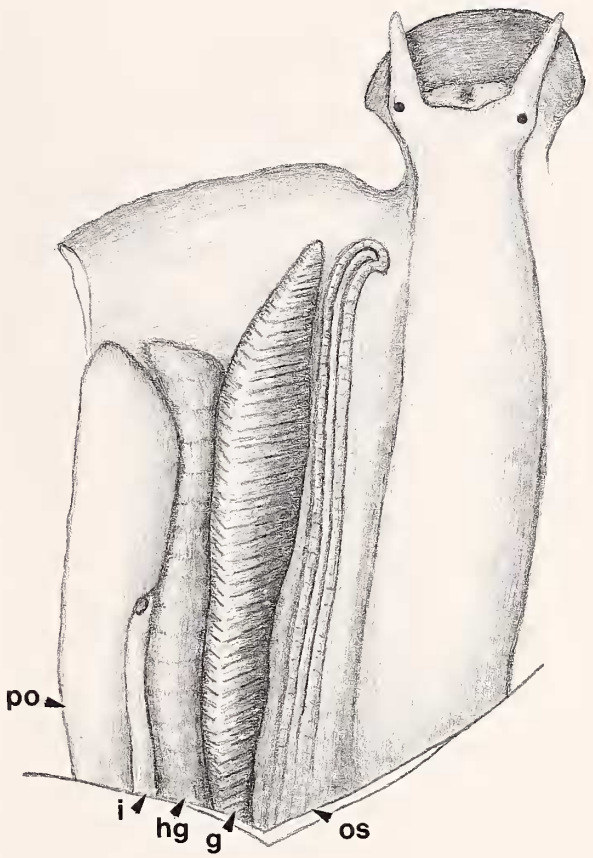

Figure 5

Diagram of the reflected mantle cavity. Key: g = gill, hg = hypobranchial gland, i = intestine, os = osphradium, po = pallial oviduct.

whorl. In fixed material the gland becomes very dark, almost black, and the pigment takes on a crystalline appearance. The pigment stains the edge of the gills next to the hypobranchial gland, and the mantle anterior to the hypobranchial gland is speckled brown. The osphradium is composed of three simple ridges that run along the ventral edge of the gill and curve ventrally at their anterior end. The two external ridges have evenly spaced transverse striations and connect to each other around the anterior edge of the middle ridge (Figure 5). The central ridge is smooth. The distal portion of the intestine sits between the pallial oviduct and the hypobranchial gland but extends only halfway to the aperture (Figure 5). The columellar muscle also extends the length of the first whorl. The heart and kidney lie just posterior to the mantle cavity. The heart is typically monotocardian and is surrounded by the flat round kidney.

Alimentary System. The most prominent feature of the alimentary system is the acrembolic proboscis. The proboscis is surrounded by a layer of circular muscles which serves as an attachment for a complex assortment of retractor and extensor muscles. In the fully extended position, the jaws lie at the distal tip of the proboscis with the radula immediately behind them. In the fully retracted position, the jaws are at least a third of the way back from the opening into the proboscis introvert (Figure 6). The two large triangular jaw plates are surrounded by a muscular sheath (Figure 7A, B). Each jaw is made of many individual lamellae that can be seen easily with light microscopy to extend across the width of the jaw. The distal edge of each lamella projects through the surrounding tissue producing a row of fine serrations along

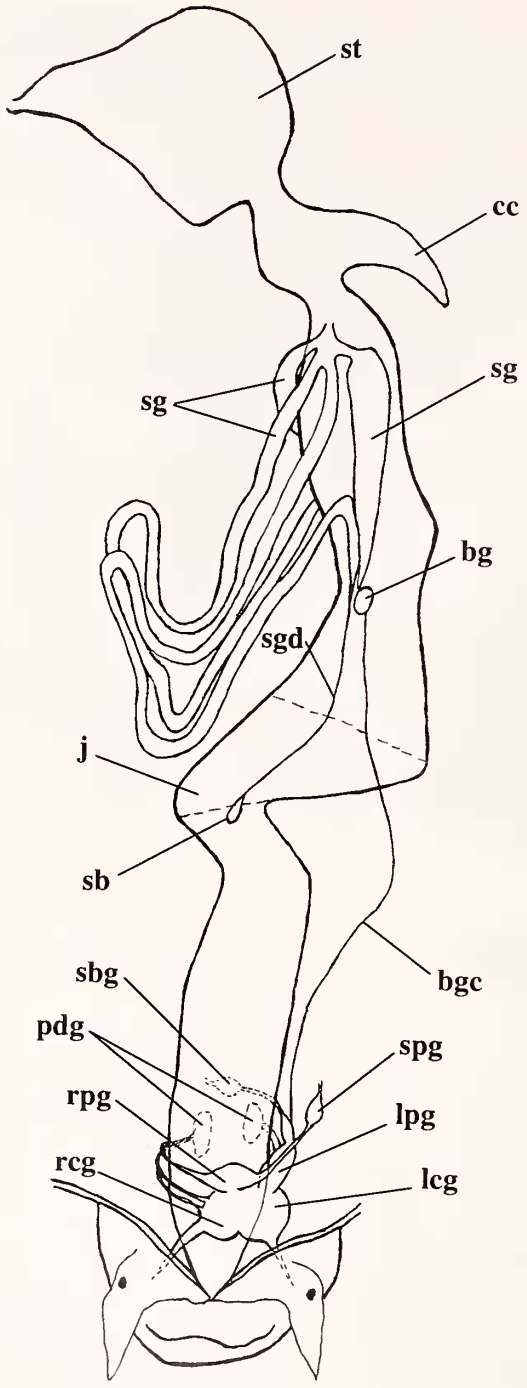

Figure 6

Diagram of the proboscis and salivary glands with the overlying tissue and the complex assortment of retractor muscles removed. These structures are reflected to the animal's left so that a lateral view of the proboscis is presented. The nerve ring is also dissected from the connective tissue in the head, but the ganglia are portrayed in situ. Key: bg = buccal ganglion, bgc = buccal ganglia connectives, cc = cardiac caecum, j = jaws, lcg = left cerebral ganglion, lpg = left pleural ganglion, pdg = pedal ganglia, rcg = right cerebral ganglion, rpg = right pleural ganglion, sb = stylet bulb, sbg = subesophageal ganglion, sg = salivary glands, sgd = salivary gland ducts, spg = supraesophageal ganglion, st = stomach.

the edge of the jaw (Figure 7C). Immediately anterior to the jaws, embedded in the muscular wall of the oral tube are two small muscular stylet bulbs (Figure 6). Two fine cylindrical stylets project from the bulbs into the lumen of the proboscis. Ducts embedded in the wall of the esophagus run posterior from these bulbs to the salivary glands.

Immediately posterior to the jaws is the divided odontophore and radula. The radula is as wide as it is long, has no central rachidian tooth, and is only slightly curved in the dorsal-ventral plane. The teeth vary in morphology; some have a hook on the distal end, whereas others end in a point (Figure 7D). There are a variable number of serrations along the edge proximal to the hook. The medial teeth are not as densely packed and have a more needlelike appearance, whereas the lateral ones are densely packed and more clearly hooked (Figure 7D).

There are two pairs of tubular salivary glands (Figure 6). The outer pair are relatively thick, slightly flattened, and have a rough appearance. Anteriorly, they attach to the buccal mass at the level of the buccal ganglia, posterior to the jaws and radula, but I was not able trace their ducts. The inner pair are long, smooth, thin coiled tubes that loop along the esophagus and attach near the buccal ganglia. Their ducts then run along the proboscis to the stylet bulbs. Posteriorly, all four glands are attached to the posterior proboscis in the same place.

Posterior to the salivary glands, the posterior constriction of the esophagus connects to the cardiac pouch and the stomach. The stomach's thin walls are covered internally with numerous shallow folds. It is not cuticularized and there is no style sac. The intestine is simple and empties into the back of the mantle cavity.

Nervous System. The nervous system is similar to other typical streptoneurous gastropods. There are two distinct buccal ganglia at the base of the salivary glands (Figure 6). When the proboscis is retracted, they lie far back in the first whorl. The nerve ring lies in the animal's head, directly behind the tentacles (Figure 6). All the ganglia, except the sub-esophageal ganglia lie on the dorsal left side of the esophagus, behind the left tentacle. The cerebral ganglia are large, round, and flattened, and the commissure is very short. There is a distinct nerve running from each cerebral ganglion into the tentacle on the same side. The slightly elongated pleural ganglia lie behind and to the left of the cerebral ganglia and are tightly connected to them. The right pleural ganglion is oriented transversely, whereas the left one has an anterior-posterior orientation. The subesophageal ganglion lies against the

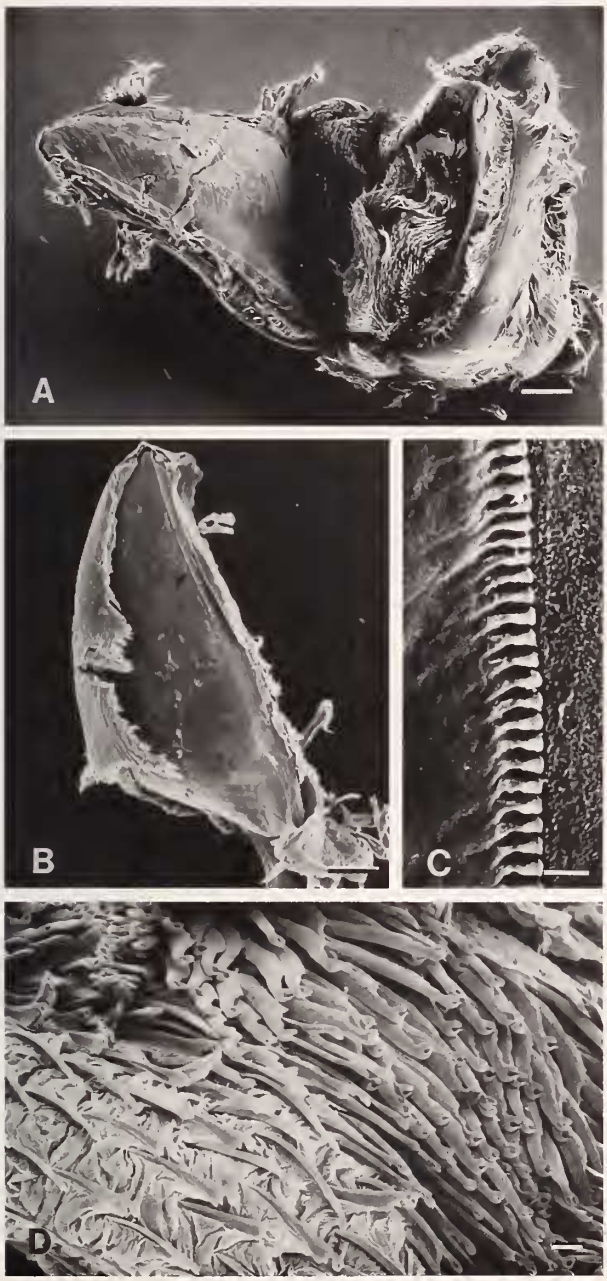

Figure 7

SEM of the jaws and radula. A. View of the buccal cavity through the jaws that have been split open along the dorsal muscular connection. The natural opening is along the ventral edge (bottom in this view). The radula is at the base of the jaws. Scale bar = 100 μm. B. One jaw removed from the buccal mass. Arrow indicates the serrations enlarged in C. Scale bar = 100 μm. C. Close-up view of the serrations where the underlying jaw extends from the tissue. Scale bar = 5 μm. D. The radula. The area with sparse teeth is more medial on the radula. Scale bar = 10 μm.

columella muscle under a thin layer of connective tissue on the far right side on the muscle. It does not connect to the right cerebral or pleural ganglia. The supraesophageal ganglion is connected to the right pleural ganglion by a short connective and lies to the left of and slightly below the other ganglia in the connective tissue just to the left of the columella muscle. The two oval pedal ganglia are connected to the rest of the nerve ring by a large number of individual connectives that run from the pleural and cerebral ganglia. They are deeply embedded in the foot musculature.

Reproductive System. The gonad occupies the dorsal two-thirds of all the posterior whorls. In the female the pallial oviduct occupies the dorsalmost one-third of the body whorl. It is large, glandular, and slightly flattened. It is not differentiated into distinct regions and is open along its entire length on the dorsal side. Males are aphallic and produce large spermatozeugmata.

DISCUSSION

Development

The development of *N. tincta* is unusual because it combines characters that were previously thought to be specific to caenogastropods with characters that were thought to be specific to heterobranchs (Table 3). *Nitidiscala tincta* develops a distinct polar lobe and embryonic kidneys that are characteristic of caenogastropods. However, the larva has a dark pigment spot that appears to be the same as a larval heterobranch PMO, and the larval shell is hydrophobic. None of these characters has been reported in non-heterobranch, non-caenogastropod snails, so it is unlikely that these characters are symplesiomorphies.

Epitoniid egg capsules are typically sediment- (sand or mud) covered mucus capsules that are strung together along a mucus thread (Robertson, 1983a b). However, *Habea inazawai* Kuroda, 1943, capsules have a reticulate surface and are not covered with sediment (Habe, 1943). There are no previous descriptions of early development to compare with my observations. The few accounts of epitoniid larvae describe them as small and having a prominent PMO (Habe, 1943; Thorson, 1946). All observed epitoniid larvae have right-handed shells, which brings into question the observation that *Couthouyella* is heterostrophic (Gosliner, 1981).

Little is known about the embryology of other pteneoglossan gastropods (with the possible exception of some viviparous janthinids). Natarajan (1957) observed that hatching larvae of *Janthina* are small and have a distinct PMO but no eyes or tentacles. Cipriani et al. (1994) observed embryonic kidneys in cerithiopsid embryos. The larvae have a distinct dark spot in the viscera, which is not, however, in the appropriate position for a PMO (R. Cipriani, personal communication). The later stages of cerithiopsids and triphorids are, however, well known

Table 3

Distribution of developmental characters between caenogastropods and heterobranchs, and their condition in *Nitidiscala tincta.*

Character	Caenogastropod	Heterobranch	*N. tincta*	Reference
Cleavage	polar lobe	unequal	polar lobe	Freeman & Lundelius, 1992 van den Biggelaar & Haszprunar, 1996
Embryonic kidney	yes	no	yes	Fioroni, 1966
Larval PMO	no	yes	yes	Robertson, 1985
Hydrophobic larval shell	no	yes	yes	Collin, 1997
Left handed larval shell	no	yes	no	Robertson, 1985
Large complex larvae*	yes	no	no	Page, 1994

* Unlike the other characters, high larval complexity of caenogastropod larvae is a generalization and not a synapomorphy.

(Lebour, 1933; Fretter & Pilkington, 1970). They have a large bilobed velum, complex shell sculpture, and a distinct apertural notch. The protoconchs of janthinids, epitoniids, and eulimids are all smooth, many (comprising three to four) whorls, and have a distinctive shape that is very different from the protoconchs of cerithiopsids and triphorids.

Anatomy

The anatomy of *N. tincta* as described herein also generally agrees with the few previous descriptions of epitoniid anatomy. These descriptions are difficult to compare because different techniques were used to examine different aspects of anatomy on different species. Studies that used basic dissections include the following: Taki (1956, 1957) described the morphology of the mantle cavity and alimentary systems of five Japanese genera (*Epitonium, Amaea* H. & A. Adams, 1853, *Papyriscala* de Boury, 1909, *Gyroscala* de Boury, 1887, and *Acustiscala* de Boury, 1909); Bouvier (1886) focused on the nervous system of *Scalaria communis* Lamarck, 1822; and Warén (1980) and Gosliner (1981) commented briefly on *Couthouyella striatula* (Couthouy, 1839). Thiele (1928) made a more detailed study of *Scala magellanica* Philippi, 1845.

Studies by Taki (1956, 1957) and Fretter & Graham (1994) focused mainly on the foregut anatomy. Like *N. tincta,* individuals of *E. (Clathrus) clathrus* Linnaeus, 1758, and the five Japanese genera have a long acrembolic proboscis with a poorly demarcated buccal mass, jaw, and two pairs of salivary glands. Taki's studies show that there is, however, considerable variation in the relative size of the jaws and radulae and the development of the salivary glands. In these previous studies the jaws are figured as being smaller or perhaps the same size as the radula. In *N. tincta* the jaws are clearly larger than the radula. Thiele (1928) described stylets connected to ducts that empty into the oral cavity. Fretter & Graham (1994) also observed these in *E. clathrus,* but Taki and Bouvier apparently did not observe these structures. I observed stylet bulbs in *N. tincta* which agree with Hochberg's (1971) observation on the same species. These differences may relate to differences in feeding behavior. Unfortunately morphological descriptions are not available for the species for which the diet and feeding habits have been described.

The two prior reports of the epitoniid nervous system (Bouvier, 1886; Thielle, 1928) observed different degrees of zygoneury. In the species they studied, as in *N. tincta,* the nerve ring lies anterior in the head directly behind the left tentacle, and the pedal ganglia are connected to the rest of the nerve ring by long connectives, as are the sub- and supraesophageal ganglia. Bouvier (1886) found the sub- and supraesophageal ganglia embedded in the body wall, which agrees with the location of both against the columellar muscle in *N. tincta.* The major difference between these descriptions of the nervous system is that Bouvier (1886) and I found no indications of zygoneury on either side of the nervous system, whereas Thiele (1928) found that the supraesophageal ganglion is connected to both the left and right pleural ganglia. Neither I nor Bouvier (1886) could locate the statocysts, but Thiele (1928) described them next to the pedal ganglia.

There appear to be several errors in Fretter & Graham's (1994) comments regarding the nervous system of *E. clathrus.* They do not give a detailed description of the nervous system, but in their figure 99 they show the left cerebral ganglia located at the level of the jaws in a fully retracted proboscis, and they state that the nerve ring is at the level of the buccal ganglia in the retracted proboscis. This is not the case in any of the epitoniids that have been described in more detail. In addition, the structure labeled as "lcg" in their figure is in the same location as the stylet bulbs in *N. tincta,* which superficially resemble ganglia.

Conclusion

Although adult anatomy clearly supports caenogastropod affinities, epitoniids possess developmental characters that have previously been thought to characterize

both heterobranchs and caenogastropods, monophyletic sister groups. Further careful homology assessment, more thorough taxon sampling, and detailed explicit cladistic analysis of gastropod relationships are necessary before the homologous and homoplasious characters can be identified with any certainty. This study highlights the fact that developmental characters that were previously thought to have good congruent distribution among gastropod taxa, consistent with higher level classification, may not in fact be so unambiguously distributed when more poorly known, basal, or intermediate taxa are examined.

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